UNILATERAL STIMULATION OF BOVINE OVARIES
BY LOCAL INJECTION OF PREGNANT MARE’S SERUM
GONADOTROPHIN

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Treatment with PMSG is still the most widely used method of inducing super-
ovulation in cattle. A major problem, however, is the great variation in
response of individual animals to identical treatments (Foote & Onuma, 1970;
Mariana, Mauléon, Benoit & Chupin, 1970). This variation may, in part, be
due to differences in the absorption of injected PMSG and its subsequent
distribution to the ovary. In rabbits, intraovarian rather than systemic injection
is said to be a more sensitive test for PMSG in the Friedman pregnancy test
(Chicchini & Chiacchiarini, 1963) and intrafollicular injection of LH also
induces ovulation (Jones & Nalbandov, 1972). This report describes an
investigation of the possibility that local injection of PMSG into bovine
ovaries might produce more consistent supervolulatory responses than do
systemic injections.

As outlined in Table 1, thirteen heifers of mixed breeding were used in
fifteen experiments. Two heifers were used a second time (Exps 9 and 11) 54
and 90 days, respectively, after excision of the treated ovary at the completion
of Exps 1 and 6. Oestrous cycles were recorded by daily observation of behaviour
in the absence of a bull. Ovaries were injected with various doses of PMSG
(Gestyl, Organon) at laparotomy on Day 16 of the cycle (day of oestrus =
Day 0) in all but two cases (Exps 3 and 5) which were treated on Day 17.
Laparotomy was carried out under general anaesthesia (halothane in N2O/O2)
and injections were given either to the ovary containing (ipsilateral to) the
CL or to the contralateral ovary. Four routes of injection were used: intra-
stromal (i.s.), sub-cortical (s.cort.), intra-arterial (i.a.) and intrafollicular
(i.f.). Injections by the first three routes were made with a 25- or 26-gauge
needle, the required dose of PMSG being dissolved in 1-0 ml of 0·9% NaCl.
For the first three routes, doses were injected: (1) 1·5 cm into the ovarian
stroma (i.s.), (2) just below the surface at two sites (s.cort.), or (3) into the
convoluted ovarian artery about 5 cm from the hilus (i.a.). For i.f. adminis-
tration (Exps 14 and 15), a microsyringe and 27-gauge needle were used to
inject each of five follicles (5 mm in diameter) in each treated ovary with 20 i.u.
PMSG in 10 µl saline by the technique of Smeaton & Robertson (1971). In
Exps 14 and 15, the CL was enucleated at the time of surgery but left in the

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Table 1. Effects on bovine ovaries of unilateral injection of PMSG

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>PMSG treatment</th>
<th>Ink injection</th>
<th>Ovary* treated</th>
<th>Treated cycle</th>
<th>Ovarian stimulation</th>
<th>Untreated</th>
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<tbody>
<tr>
<td></td>
<td>Route</td>
<td>Dose (i.u.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Intrasstromal</td>
<td>400</td>
<td>+</td>
<td>C</td>
<td>±</td>
<td>19·5</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>+</td>
<td>I</td>
<td>-</td>
<td>-</td>
<td>23·5</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>-</td>
<td>C</td>
<td>±</td>
<td>21</td>
<td>3 (0)</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>-</td>
<td>I</td>
<td>±</td>
<td>22</td>
<td>4 (4)</td>
</tr>
<tr>
<td>5</td>
<td>1200</td>
<td>+</td>
<td>C</td>
<td>+</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1200</td>
<td>+</td>
<td>I</td>
<td>+</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Subcortical</td>
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<td>-</td>
<td>C</td>
<td>+</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>800</td>
<td>-</td>
<td>I</td>
<td>+</td>
<td>25</td>
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</tr>
<tr>
<td>9</td>
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<td>400</td>
<td>-</td>
<td>I†</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>400</td>
<td>-</td>
<td>C†</td>
<td>+</td>
<td>24</td>
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<tr>
<td>11</td>
<td>400</td>
<td>-</td>
<td>I†</td>
<td>+</td>
<td>24</td>
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<tr>
<td>12</td>
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<td>-</td>
<td>I</td>
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<tr>
<td>13</td>
<td>800</td>
<td>-</td>
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<td>±</td>
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<td>14</td>
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<td>C§</td>
<td>±</td>
<td>19</td>
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<tr>
<td>15</td>
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<td>-</td>
<td>C§</td>
<td>±</td>
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</table>

* Ipsilateral (I) or contralateral (C) to the CL present at the time of treatment. † Figures in parentheses = ova recovered by flushing. ‡ Only remaining ovary. § Corpus luteum enucleated. L = luteinized.
abdominal cavity in order to synchronize treatment and CL regression more precisely (see Whitmore, Ginther & Casida, 1971). Ovarian changes were determined at a second laparotomy 9 to 18 days after treatment, between Days 3 and 12 of the following cycle. Signs of stimulation were assessed from written and photographic records supplemented by flushing the genital tract for ova (Exps 3, 4, 6, 11 and 12) and histological examination of excised, formalin-fixed ovaries (Exps 1, 2, 4, 6, 10 and 11).

To see whether the follicles responding to PMSG were the largest ones present on Day 16 or were smaller immature follicles, the i.f. injection technique was used to mark the largest follicle in the treated ovary with Indian ink in Exps 1, 2, 5 and 6. These follicles, 11 to 22 mm in diameter, were injected with 6 to 7 µl marking fluid.

After treatment, as shown in Table 1, full standing oestrus (+) was observed in eight heifers, two showed no signs of oestrus (−) and five heifers showed oestrous symptoms short of actual standing (±).

Signs of follicular stimulation were confined to the treated ovary in all five intact heifers that responded to 400 or 800 i.u. PMSG either i.s. or i.a. (Table 1, Exps 1 to 4 and 10). No difference was observed between treated ipsi- and contralateral ovaries. The two unilaterally ovariectomized heifers (Exps 9 and 11) also responded to 400 i.u. PMSG i.a. The two heifers receiving 1200 i.u. PMSG i.s. (Exps 5 and 6) showed a bilateral response. The ineffectiveness of 800 i.u. PMSG i.a. (Exps 12 and 13) may have been due to misplaced injections because in neither case had arterial spurting followed needle withdrawal. Such technical difficulties, and the fact that only about 20% of ovarian blood flow passes through the stroma in sheep (Mattner & Brown, cited by Goding, Baird, Cumming & McCracken, 1971), suggest that i.a. injection has no advantage over the simpler i.s. route. Sub-cortical and i.f. injections, however, failed to stimulate follicular development. Failure of i.f. injections was more probably related to follicular immaturity than to inadequate PMSG dosage because each follicle received approximately 1·25% of a systemically effective dose whereas rabbits ovulate after i.f. injection of less than 0·1% of their systemically effective dose (Jones & Nalbandov, 1972). Adhesions from CL enucleation precluded direct inspection of untreated ovaries in cows receiving i.f. injections but subsequent cycles of normal lengths indicated that they had ovulated (Table 1).

Only three treated heifers were judged to have two or more ovulations. One (Exp. 6) had received 1200 i.u. which approaches a systemically effective dose and diagnosis in the second (Exp. 4) could not be confirmed by recovery of tubal ova. Although local injection of PMSG does not therefore seem a feasible means of superovulating cattle, it is of interest that unilateral stimulation can be induced with low doses of gonadotrophin which presumably binds immediately to ovarian receptors. Three of the four ink-marked Day-16 or -17 follicles regressed. This was probably not an inhibitory effect of i.f. injection because ovulation followed in five out of six mature follicles that were similarly marked in a cow (not listed in Table 1) in oestrus following systemic PMSG treatment. The one Day-16 marked follicle to show signs of responding to PMSG (Exp. 1) enlarged to 20 mm but neither ovulated nor luteinized.
Ovulation may have been inhibited by inadvertent injection of ink between granulosal and thecal cell layers because the same had occurred in the one follicle marked at oestrus that failed to ovulate but grew to 30 mm. The partial response of the largest follicle in Exp. 1 was associated with a relatively short treated cycle (19.5 days). Dufour, Whitmore, Ginther & Casida (1972) have shown that the largest follicle detected more than 3 days before oestrus regresses and does not respond to endogenous gonadotrophins. The limited data of the present experiments suggest that the same is true of response to exogenous PMSG.

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REFERENCES


